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Structure of the Binding Domain of Tetanus Neurotoxin in Complex with Doxorubicin

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Beamline(s): X12C

Introduction: Clostridial neurotoxins comprising the seven serotypes of botulinum neurotoxin and the tetanus toxin are so far the most potent neurotoxins known to affect humans. They cause spastic and flaccid paralysis in humans. Biochemical, electrospray ionization mass spectroscopy (ESI-MS) and computation docking studies have shown that doxorubicin, a well-known DNA intercalater, binds to these neurotoxins. Here we present the crystal structure of the C-fragment of tetanus toxin complexed with doxorubicin to identify the binding site for the drug and its interaction with the protein molecule.

Methods and Materials: Crystals of the complex were prepared by soaking native crystals in the mother liquor containing doxorubicin. Crystals of tetanus toxin C-fragment were obtained by the sitting drop vapor diffusion method with PEG 4000 as the precipitant and MgCl2 as an additive in imidazole buffer at pH 6.5. This doxorubicin soaked tetanus neurotoxin C-fragment crystal was flash frozen and mounted in the liquid nitrogen stream. X-ray diffraction data were collected at the X12C beam line of the National Synchrotron Light Source, Brookhaven National Laboratory. The crystals belong to the orthorhombic space group $P2_12_12_1$ with the cell parameters a = 66.61, b = 78.80, c = 90.05 Å. The data were collected using MARMAD and processed using DENZO/SCALEPACK. The drug molecule was identified from the difference Fourier map and the structure was refined using CNS. The final R factor and free R were 0.22 and 0.28, respectively for the complete 2.8Å resolution data.

Results: Doxorubicin binds in a site that has been identified as the binding site for gangliosides. Doxorubicin stacks against the conserved residue Trp 1289 and interacts with another conserved residue, His 1271. This binding pocket and the interactions are similar to BoNT/B-doxorubicin complex. Results from this study will enhance the knowledge in designing the potential molecules for inhibiting neurotoxin binding to the membranes.

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